WILLIAMS

Chemical Study of the Water Extract of Meat

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A Chemical Study of the Water Extract of Meat

...BY...

ELRICK WILLIAMS

THESIS

FOR THE

Degree of Bachelor of Science in Chemistry

IN THE

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Elvie Williams

ENTITLED A Chemical Study of the Water Extract of Meat

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

OF Bachelor of Arts.

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5548

A Chemical Study of the Water Extract of Meat.

The detailed analysis of meat extracts show that a considerable portion ranging from three to forty-four percent are estimated indirectly, usually by difference. Either of two reasons might account for this, the constituents are not sufficiently well known or the methods employed for their determination are inaccurate.

The object of my work has been to separate if possible, and estimate these constituents.

For more than forty years after meat extract was described by Proust (1801), little investigation of value was done. In 1847 Liebig whose name stands pre-eminent in the early investigation of meat extract, published the results of his work. This date marks the beginning of anything like accurate and valuable investigations. He attributed a relatively greater nutritive value to the nitrogenous material than is given to-day.

His original method of preparing meat extract consisted in soaking the finely hashed meat in eight times its weight of cold water, filtering off the insoluable portion,largely fibre, and then heating the filtrate to coagulate the albumin. After filtering again the filtrate was evaporated to a syrup. In practive, however, a higher temperature was used for extracting the soluble portions. In his paper (1) he adds a table which gives the constituents and their relative quantities as then determined.

From 1000 grams of beef:-

Soluble in Cold Water 60 gr.	Coaguable Albumins 29.5.
	In Solution 30.5.
	Gelatin 6.0.
Insoluble in Water 170 gr.	Insoluble Proteids 164.0.

Fat	20 gr.
Water	750 gr.
	1000 gr.

Among the constituents separated and studied he gives:- creatin, creatinin, sarcosine, inosinic acid and inorganic constituents. From 100 pounds of flesh of a lean old horse, he obtained 36 grams of creatin and 86 poubds of ox flesh gave 30 grams of creatin. No figures for the other constituents were given.

The above method described by Liebig was not applicable on a commercial scale and hence has been supplanted by others. Nearly every firm has its own method which is kept as nearly secret as possible. In general, however, the process is as follows. (2)

The beef freed from fat and bones is cut up into small pieces after being thoroughly washed. It is then placed in vats with an equal weight of water and one-fifth its weight of hydrochloric acid and cooked fifteen to twenty hours. This material is ground up again and cooked for fifteen to twenty hours more. After neutralization with sodium Carbonate the residue is evaporated to the proper consistency.

Some manufacturers allow the finely cut beef to stand a few hours in contact with cold water, then boil the liquid for a time and finally evaporate down the extract obtained on filteration in vacuum pans. In some establishments steam under pressure is used for extracting the meat, the mass being subjected to powerful hydraulic compression and the liquid obtained dried in vacuo. Since about thirty-five pounds of meat are necessary to produce one pound of extract, it is evident that a large percent of the nutrient material of the original meat is excluded. Men have come, however, to recognise extract of meat as it should be recognised; that is, not as a nutrient but as a stimulant. This property is due as Allen (3) says to the saline and extractive matters of the meat.

In forming an opinion of the value of a meat extract the attention is turned to the following constituents:-

I. Proteids in the form of albumin, gelatin, albumoses, peptones, and meat fibre.

II. Other nitrogenous compounds especially the meat basis as creatin and creatinin.

III. Non nitrogenous organic matter.

IV. Mineral constituents or ash.

V. Water.

That it is important to estimate the water may be seen from the fact that extracts of various makes and description vary in the water content from 10 or 12% to 90 or 92%.

Hehner (4) when investigating the method of proteid precipitation by means of alcohol obtained the following percentages of water. Liebig's extract 18.70%. Concentrated beef tea, English 41.93%.

Russian 24.56%. Commercial Essence of Beef, English, 92.32%. South African 87.55%.

The mineral constituents vary, of course, with the concentration of extract. (5) Hehner's table shows the following:-

Brand & Co's. Essence of Beef 1.0%. Vitalia Meat Juice 6.65%. Bovril for Invalids 16.50%. Bothwicks Boullion 17.93%. Liebig Co's. Extractun, Carnis 23.51%. Armour's Extract of Meat 29.36%.

The non-nitrogenous organic matter including glucose, milk, sugar, fat, inosite and glycogen have not received much attention in the past but of late they are coming into more prominence. In most cases where these constituents have been estimated, they appear under the head of non-nitrogenous extractive matters and are obtained in many cases by difference. That is, the sum of the percentages of the constituents determined does not equal 100, and as the methods for estimating the non-nitrogenous organic matter are not well developed the difference between 100% and the sum obtained by actual analysis is often attributed to these extractive matters.

The following table illustrates this point:- (6)

Constituents.	Liebig Co's. Extract.	Bovril Fluid Beef Seas-oned.	Bovril for In-valids.	Brand & Co's. Beef Bouil-lon.	Esco Beef Bouil-lon.	Vimbos.	Viking Beef Es-sence.
Water.	18.35	38.10	21.16	36.27	52.43	34.93	90.68.
Insoluble Proteids, meat fibre, etc.	Trace	8.40	8.47	3.48	0.56	7.23	none.
Soluble Proteids and Gelatin.	9.45	3.81	8.19	5.73	7.10	4.28	3.63.
Flesh Bases(Nx3.12).	29.67	12.04	16.13	19.34	5.90	12.60	1.85.
Non-nitrogenous Extractive Matters.	18.85	19.75	29.23	19.75	13.62	23.61	2.41.
Mineral.	23.68	17.87	16.82	15.07	20.39	17.35	1.43.
Total.	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The fact that in each case the total equals 100%, would appear from our previous experience to show that some of the constituents were determined by difference. Since the methods employed for determining the extractive matters are the least reliable even if these constituents are determined at all, we are somewhat justified in concluding that these percents were taken by difference. In a table by Gautier given later, we notice that inosite and glycogen have been estimated directly and equal 7.6% of the total solids of the extract from a liter of broth. He gives however, various extractive matters as eq al to 14.13% which is as high as that obtained from "Esco" beef juice by Tankard. If we add to this the 7.6%, usually not determined directly, we have 21.73% which is approximately the percent ordinarily determined by difference.

The results of Mr. Thos. Macfalane (7) of the Canadian Inland Reven-

ue department are of interest here.

Constituents	%	%	%	%
Water.	21.900	20.800	25.200	54.600.
Ash.	22.200	17.600	33.000	11.100.
Fat.	1.070	1.020	0.230	0.940.
Insoluble Proteids.	3.575	3.431	0.813	-------
Proteids p'p'td. by Br.	6.290	5.425	9.456	2.006.
Flesh Bases.	18.876	24.845	8.867	8.867.
Undetermined by Diff.	26.089	26.879	22.434	22.487.
Total	100.000	100.000	100.000	100.000.

Here we see the undetermined constituents range from 22 to 26%. The first two analyses are of a meat extract and the last two are of beef juice. We shall refer to this problem again.

Probably the greater part of the work done on Extracts of meat has had to do with the separation of the proteids and the compounds related to them. Of the use of alcohol as a precipitating reagent, Mallet (8) says that the proteids precipitated by it vary in character and amount with the quantity and strength of the alcohol used. Nearly all simple amids and amido acids are either insoluble or so slightly soluble in it that it is almost impossible to wash them from the precipitated proteids. This method has been used for a number of years in the laboratories of manufacturers for distinguishing between the forms of nitrogen constituents of meat extracts. Allen (9) says it was only a rough estimation. Hehner (10) described a method in which alcohol was used and arrived at the conclusion that alcohol should not precipitate much more than 25% of the total dry matter of meat essence nor more than 40% from beef tea. The following table

is from Hehner:

Description.	H_2O.	Solids.	Alcohol Ppt.	Ash.	P_2O_5	N.
Liebigs Ext.	18.70	81.30	5.16	23.38	6.07	7.94.
Beef Tea-English.	36.94	63.04	27.40	4.36	1.16	8.25.
Beef Tea-English.	41.93	58.07	25.30	4.92	1.10	7.52.
Beef Tea-Russian.	24.56	75.44	35.40	6.72	0.95	9.89.
Commercial Beef Essence.	89.25	10.75	3.07	1.17	0.34	1.36.

Bruylants[11] used a method of fractional precipitation by alcohol of different strengths. Thus he says that alcohol of 40% throws down gelatin; 80% the albumoses and 93-95% the peptones.

This method will receive further discussion later on.

Other methods have been devised. The reactions with chlorine and bromine have been used quite extensively of late. In 1897, Rideal and Stewart[12] made investigations on which they base their statement that the nitrogen of proteid materials is almost completely precipitated by chlorine. They were unable, however, to find out the composition of the precipitate. Mulder used the chlorine method as early as 1840. Rideal and Stewart dried the precipitate over sulphuric acid and weighed it. This method, however, is objectionable, owing to the difficulty of manipulation Allen and Searle modified the method by using Bromine in the liquid form instead of chlorine gas. The table following shows the results obtained:

Substance.	% Nitrogen Total in Original.	% Nitrogen Ppt'd. by Br.	Nitrogen x Factor. Total.	Nitrogen x Factor. Ppt'd. by Br.	Factor.
Common Gelatin.	14.10	14.00	76.42	76.14	5.42.
Fresh egg white.	1.89	1.88	11.96	11.90	6.33.
Peptone from white of egg..	.70	.69	4.43	4.37	6.33.
Beef extractives.	.33	.004	2.11	0.03	6.33.

The use of Bromine in analysis of commercial meat extracts showed the following results:-

Extract	N in Br. Ppt.	Proteids. N x 6.3.
Liebig Co's. Extract.	1.41.	8.88.
Seasoned Bovril.	1.94.	12.22.
Bovril for Invalids.	2.64.	16.63.

The authors found that Bromine was an excellent precipitant for proteoses and peptones, and obtained no precipitate when solutions of creatin, creatinin, etc. were treated in a similar manner. They make use of the method for determining peptones after precipitation of proteoses by zinc sulphate. By subtracting the sum of the nitrogen found in the precipitate by zinc sulphate and in that by bromine from the total nitrogen, the remainder could be considered as present in the form of flesh bases.

A number of methods based on the separation of proteid material by means of saturating their solutions with metallic salts have been advanced. The principle depends upon the hydrolytic power of saturated saline solutions and any salt having this power is applicable. The method is also called the salting out process. The explanation is simple. The salt used takes up the water which holds the proteids

in solution and when this occurs, the proteids are precipitated. Ammonium sulphate has been used extensively but there is a source of error in all the NH_3 from the $(NH_4)_2SO_4$ used in the precipitation may not be driven off before determining the Nitrogen in the precipitate.

Comparative determinations by use of 80% alcohol and with ammonium sulphate are given below.

	Extract Liebigs.	Kemmerich's Extract.	Kemmerich's Peptone.	Cibil's Extract.
Total Nitrogen.	9.32	8.94	9.78	2.77.
N in Alcohol Ppt.	0.69	1.05	4.05	..61.
Corresponds to Albumoses.	4.31	6.55	25.31	3.81.
Albumoses by $(NH_4)_2SO$.	7.32	9.71	34.44	5.97.

$ZNSO_4$ has been made in place of $(NH_4)_2SO_4$ in order to avoid this error. The process is simple. Fifty cubic centimetres free from solid matter and containing about two grams of soluble proteids are acididief with dilute sulphoric acid and then saturated at the ordinary temperature with powdered ZN SO_4, the precipitate, filtered and washed with a cold saturated solution of the precipitant. The nitrogen is determined by moist combustion of filter and contents, and the Kjeldahl method being employed. Pinkus[14] suggests the use of anhydrous sodium sulphate and says that at 30°C, it serves as well as $(NH_4)_2SO_4$.

In the precipitation by Phospho tungstic acid, the method suggested by Wiley[15], the clear acqueous solution is acidified with an equal volume of H_2SO_4 (1-3) and the reagent then added until no further precipitation results.

(16)
Stutzer was the first, probably, who made use of this reagent but he held the view that meat bases were wholly unprecipitated from a strongly acidified solution by an excess of reagent. Other chemists among whom are König and Bömer believe that all flesh bases as well as the other nitrogenous constituents of meat extract are precipitated if sufficient time is allowed. Wiley evidently agrees with this view as his directions are to let the precipitate stand six days. Stutzer considered the nitrogen found in the phosphotungstic precipitate to be due to peptones, but according to analyses of other chemists this would appear untrue.

	Liebig's Extract.	Kemmerick's Extract.	K's. Peptone.	Cibil's Extract.
N. in Phosphotungstic ppt.	6.27	5.59	8.29	2.00.
N. in $(NH_4)2SO_4$ p'pt.	1.17	1.55	5.51	0.96.
Peptone (?) Nitrogen	5.10	4.04	2.78	1.04.

König and Bömer think that the flesh bases should claim a large percent of the nitrogen calculated to peptone. In order to correctly test for peptones, Bömer says to examine the filtrate from the $ZN\ SO_4$ precipitation by means of the buiret test. In many cases, the results are negative and hence he believes that, in the past, peptones have been much overestimated. Mallet has also used the phosphotungstic acid method with some modification.

Other methods of separating proteids but which have not been a success commercially are:--By dialysis, decomposition by nitrous acid, interaction with $K_2\ Mn_2O_8$, interaction with sodium hypobromete, cupric hydroxide, carbolic acid, formic aldehyde, hydro-zoic acid in acqueous solution, and by chloroform.

Even those methods which are used commercially at present yield

results which are not concordant when practiced by one with a limited experience. The above discussion covers fairly well that material ordinarily known as meat extract. We have still to consider beef broth and cold water extracts.

There should be quite a clear distinction between beef extract and beef broth. The former is a term applied usually to commercial extracts or fluids prepared in different ways which are trade secrets. Beef broth is a term applied to the water solution obtained by boiling meat in the manner usually employed by the house wife. Even the unskilled person knows that a richer broth is obtained under certain conditions than under others.

Among the first to make a scientific investigation of this subject was Vogel.(17) He showed that a richer broth could be obtained by placing the meat in cold water and raising the temperature gradually than by placing it immediately into very hot water. The reason for this is that the high heat coagulates the proteids near the surface and prevents a large amount of soluble material from escaping into the broth. This coagulation does not occur when the water in which the meat is placed is heated gradually. The question of the quantity of material cooked out in broth began to interest other chemists and since Vogel's investigation in 1884, considerable has been done along this line.

Probably the next to take up this work was Nothwang.(18) Thudichum(19) was also among the first to investigate the losses involved in cooking meat. He states that from one-fifth to one-third of the weight of the meat is cooked out by boiling or roasting. In 1893 Konig(20) reported several analyses showing the loss involved in boiling

and roasting meat. His was an indirect method as he gave the analysis of the original meat before and after cooking. In 1897, Gautier (21) in an article on the value of the importation of refrigerated meats and meat broths. He boiled a kilogram of meat a long time and obtained two and one-half liters of broth which yielded from thirty-three to forty grams of dry residue. He says the residue usually amounts to twenty or twenty-two grams per liter, depending on the age of the meat, the time used in boiling and whether or not salt was used. In the latter case, the proteids are partially peptonized and brought into solution. He gives a table showing the approximate composition of the extract of a liter of broth. The percentage composition is calculated to the total solids.

Constituent.	Grams.	%
Peptones & propeptones.	5.3.	28.91.
Heloses (gelatin).	2.6.	14.13.
Creatinic bases.	1.8.	9.78.
Other bases.	0.2.	1.08.
Inosite and Glycogen.	1.4.	7.60.
Various Extractive Matters.	2.6.	14.13.
Mineral Salts.	4.5.	24.45.
Total.	18.4.	100.08.

As far as is known the methods employed in this investigation have not been published.

In 1899 Dr. Grindley and Mr. Porter (22) took up the study of meat broths. They found that when placed directly in boiling water, the average loss, eight experiments, was 41.6% of the total weight of the meat. In three experiments in which the meat was placed in cold

water and heated up gradually the loss was 42.4%. Regarding the loss of various constituents of meat, the following table will be of interest.

Constituents	In Hot Water.	In Cold Water.	Avg. of 48 Tests.
Water.	54.3.	54.3.	48.0.
Total Nitrogenous Matter.	5.1.	5.0.	4.4.
Fat.	13.1.	20.7.	13.5.
Mineral.	51.1.	52.0.	45.9.

The composition of a broth analized by Dr. Grindley and Mr. Porter gave the following results, calculated to the total solids. Creatin and other bases 27.7%; Mineral matter 23.8%; Extractives 14.4%; Albumin,peptones, gelatinoids, etc. 13.6 %. This leaves 20.5% of the total which has not been estimated. In almost every case where a similar analysis has been made, there is a certain portion, ranging from a few percent to as high as forty-four, which escapes the ordinary methods of analysis. In the fall of 1900, Emmett (23) took up the study of meat broths with the purpose of isolating and estimating if possible, the material otherwise estimated by difference. Some of the problems he investigated are:- The proper factor for calculating the flesh bases. Hehner (24) used 6.25 while Stutzer (25) preferred 3.12, which although he thought too low he considered more nearly correct than 6.25. Emmett's investigation showed that while the use of these different factors gave results varying considerably, yet it could in no way account for so large a percentage of undetermined material.

He also studied the possibility of water being held mechanically by the total solids and the possibility of errors due to the

methods of analysis. He concluded that neither of the above reasons was sufficient to account for the difference and states in his resume that it is due at least in part to non-crystallizable organic matter of alkaloidal properties containing a small percentage of nitrogen.

The third group of extracts, if they may be so called, includes those obtained by treating the hashed meat with water at the ordinary temperature. Liebig as already stated, treated meat in this way. From 1000 grams of ox flesh, he obtained 60 grams of soluble material and of this only about one-half remained in solution on heating. Gautier gives some interesting results obtained in his laboratory in 1897. From 100 parts of flesh meat, the dry extract of the portion soluble in cold water 6.92 grams, after coagulation by heat 3.86 grams. These results are a little higher than those obtained by Liebig. The relation of the water soluble portions free from bones, tendons, gristle, cellular tissue, etc. to the portions insoluble in water are as follows:- Soluble. Insoluble.

Including fat.	5.30.	15.2 .
Excluding fat.	5.37.	15.44.

I have found no further data concerning cold water extracts.

Before proceeding to the analitical work it may be of advantage to us to discuss in detail those constituents found in extracts, giving their general and specific properties. They will be taken up under the following heads:-

I. Proteids including proteoses peptones, etc.

II. Other nitrogenous constituents among which are creatin, creatinin, and other flesh bases.

III. Non-nitrogenous constituents as Inosite, Glycogen- etc.

IV. Mineral Constituents.

Proteids are highly complex, nitrogenous organic compounds, the correct formulae of which are not known. Some of their general properties are:- They discompose on heating, with evolution of ammonia. All true proteids contain sulphur which may be detected by boiling with strong NaOH and a few drops of lead acetate, Pb S is formed. Solutions of proteids have a laevo rotary effect on polarized light. Neutral or faintly acid solutions of proteids (not peptones or proteoses) yield a precipitate when heated to boiling for a few minuites. Neutral or alkaline solutions (not peptones) coagulate on addition of dilute mineral acid. They are more or less completely precipitated by saturation with neutral salts. Most salts of heavy metals separate proteids from their solutions as a compound of the proteid with the oxide of the metal added. Silver nitrate, basic lead acetate, mercuric acetate, copper-sulphate, ferric acetate, and mercuric chloride act in this way. The proteids can be set free again by use of hydrogen sulfide. They are completely precipitated by boiling with hydrated oxide of lead, copperm and zinc. Most of the proteids are precipitated by potassium mercuric iodide in HCl solution. By addition of picric acid to an acetic acid solution of proteids, they are very completely precipitated, potassium ferrocyanide gives a white floculent precipitate. Several color reactions for proteids are characteristic. Fuming HNO_3 destroys solid proteids, a weaker acid produces a bright yellow solution due to the formation of xantho-proteic acid. Millon's reagent gives a brick red precipitate. Concentrated H_2SO_4 added to a glacial acetic acid solu-

tion of a proteid gives a beautiful violet coloration and slight fluorescence Buiret test. Dilute copper sulphate added to a solution of albumin or globulin precipitates an albuminate of copper which dissolves on addition of an excess of KOH on NaOH. A deep violet coloration is produced. NH_4OH produces a blue coloration.

The most important proteids of meat extract are the proteoses and peptones. The former are products of peptic action on the more complex proteids and are intermediate between them and the peptones. They may be produced also by the hydrolytic action of acids, and alkalies and by putrefaction. Solutions of the proteoses do not coagulate on heating to boiling, are preceiptated at the ordinary temperature by nitric acid, acetic acid, and potassium ferrocyanide. The precipitate in the latter case dissolves on heating but reappears on cooling. The methods for the quantitative estimation of albumoses and proteoses have been described sufficiently in detail above. The one most commonly used is by saturation of the solution with ammonium or zinc sulphate. The final products of proteolic ferments upon proteids are the peptones. They are readily soluble in water, do not coagulate on heating and are not precipitated by nitric acid, acetic acid and potassium ferrocyanide, cupric sulphate, ferric chloride, sodium chloride nor by neutralization of their acid or alkaline solutions. Like albumoses and albumin they are precipitated in neutral or weakly acid solution by mercuric chloride, mercuric nitrate, silver nitrate, the lead acetates, tannic, picric and the biliary acids also by alcohol, phospho-molybdic and phosphotungstic acids.

Neumeister (26) says that secondary albumoses are not quite complete-

ly precipitated by phospho-tungstic acid and true peptones very incompletely. Solutions of peptones diffuse readily through animal membranes, their osmotic power, however, is comparatively feeble. They give the protein reactions, but the biuret test shows a red or pink coloration instead of blue or violet. They usually rotate the plane of polarized light to the left. The method most widely used for quantitative estimation is that of saturating the solution containing the peptone with liquid bromine. An insoluble compound is formed which may be filtered off and the nitrogen determined by the Kjeldahl process. The non-proteid nitrogenous constituents may be divided into two classes the creatinic and the xanthic bases. The creatinic bases are only slightly soluble in alcohol. They are distinguished from the xanthic bases by containing more hydrogen and by not being precipitated by copper acetate.

Creatin ($C_4 H_9 N_3 O_2$) is a feeble base, white and opaque, which crystallizes with one molecule of water in colorless rhombic prisms. Crystals melt at 100°C. Very soluble in hot water and in seventy-five parts of cold water. Slightly soluble in dilute alcohol, less so in absolute, insoluble in ether. The acqueous solution has a bitter taste and is neutral to litmus. It forms crystallizable monobasic salts with acids and unites with certain salts as $ZN Cl_2$. Solutions of creatin heated above 30°C lose water and creatinin is formed. When boiled with $Ba(OH)_2$ it is decomposed into sarcosine, urea, and methyl hydantoin. It is not precipitated by phospho-molybdic acid in HCl solution, but sodium phospho-molybdate precipitates it as does also $ZN Cl_2$ in presence of HCl and alcohol. It reduces Fehling's solution but no Cu_2O is formed. No precipitate is

produced by iodine and potassium iodide solution. All the creatinic bases give precipitates with silver nitrate and mercuric chloride. Neubaner's[27] method for separating creatin is al follows:-

The water solution was precipitated with lead acetate and the filtrate treated with H_2S to remove excess of lead. Evaporated until on cooling crystals begin to separate and then added two or three volumes of 88% alcohol. The crystals were collected on a filter and weighed if desired. They are yellow at first, but may be obtained colorless by recrystallizing. Stödeler's process consists of first extracting the finely divided meat with twice its volume of alcohol, evaporating off the alcohol and adding basic lead acetate. The process is then continued as above.

Creatinin ($C_4H_7N_3O$) is a strong base which crystallizes in brilliant prismatic crystals, which are readily soluble in cold water and in 120 parts of alcohol. Creatinin is precipitated by the following reagents. Sodium phospho-molybdate in acid solution, picric acid, zinc chloride, mercuric chloride but neither by Brouchardts reagent (I plus KI) nor Selmis' reagent (Fe_2Cl_6 plus K_3FeCN_6). Oxidizing agents produce methyl guanidine and $Ba(OH)_2$ methyl hydantoin. It is completely decomposed by boiling with caustic alkaliss. It has marked reducing properties, including reduction of Febling's solution. Creatinin is estimated by converting it into the sparingly soluble double compound with $ZnCl_2$. This has the formula $(C_4H_7N_3O)_2ZnCl_2$. Iso, xantho-, cruso-, and amphi creatinin and a few other bases resemble creatinin in properties but need not be taken up here.

The xanthic bases resemble uric acid and have basic and acid

properties. When heated with Alkalies, their nitrogen is converted into hydro-cyanic acid.

Xanthine ($C_5H_4N_4O_2$) when freshly separated from its solutions has the appearance of white amorphous granules. It is soluble in 14,000 parts of cold water and 1160 parts of boiling water. It is decomposed at 156°C with formation of ammonium cyanide, carbon dioxide, formic acid and glycocoll. Nascent hydrogen converts Xanthine into sarcine. Solutions of alkalies dissolve xanthine and it may be precipitated again by addition of acid. When heated with silver salts in ammonical solution it reduces the silver to the metallic state. Pure warm nitric acid dissolves xanthine without producing gas and when the solution is cautiously evaporated to dryness, a colorless nitrate is left, which is not rendered purple by ammonia. These reactions serve to distinguish it from urea.

Hypoxanthine or sarcine is a white crystalline powder which has the formula ($C_5H_4N_4O$). It is more soluble in water than xanthine. When oxidized with permanganate xanthine is produced but nitric acid does not give the same result. Its hydro-chloride crystallizes in bright prisms. It is precipitated from its acid solution by phospho-molybdic acid but not by picric acid. Pure sarcine does not give the murexide test. It is separated from meat extract by converting it into the nitrate, then the picrate, and adding $AgNo_3$ to the boiling solution. The salt thus obtained, is decomposed with HCl and the solution extracted with ether. The hydrochloride of hypoxanthine remains in solution and may be precipitated by sodium carbonate.

Adenine ($C_5H_5N_5$) has been extracted from the glandular tissues

of young animals but has not been found in the acqueous extract of muscle.

Guanine ($C_5 H_5 N_5 O$) has been found in very small quantities in muscle. It is a colorless usually amorphous powder, which may be crystallized by allowing its ammonical solution to evaporate spontaneously. Easily soluble in mineral acids and alkalies. It acts like xanthine in the nitric acid test but when heated with alkalies gives a strong bluish violet color. A yellow precipitate of silky needles is produced by adding cold picric acid solution to a warm solution of quanine hydrochloride. Orange crystals sparingly soluble in water are formed on addition of platino chloride. The sulphate forms long yellow needles. It is precipitated by mercuric chloride, potassium ferrocyanide and potassium chromate.

Carnine ($C_7 H_8 N_4 O_3$) was separated from meat extract by Weidel. It is a white crystalline base having a neutral reaction and bitter taste. Difficultly soluble in cold water, insoluble in alcohol and ether, but dissolves easily in hot water. Bromine or nitric acid converts it into hypoxanthine. The precipitate by basic lead acetate dissolves completely in boiling water. This distinguishes it from xanthine, sarcine and guanine; copper acetate produces a bluish green precipitate, and mercuric chloride a white one. It gives no precipitate with picric acid. The hydrochloride crystallizes in yellow needles.

Inosinic acid ($C_{10}H_{13}N_4 PO_8$)[28] is an amorphous substance which may form crystalline salts with alkalies. It is decomposed by boiling water into sarcine, trioxyvaleric acid and phosphoric acid.

Urea $C H_4 N_2 O$ and uric acid ($C_5H_4N_4O_3$) have been thought by

some authors to exist in small quantities in muscular tissues but their presence is doubtful. Traces of Taurine ($C_2H_7NSO_3$) have been found in the muscles of the horse and molluscs.

The third division of our classification, the non-nitrogenous organic matter, includes inosite, glycogen, muscle sugar, lactic acid, etc.

Glycogen $(C_6H_{10}O_5)n$ a reserve material constantly present in muscular tissue, is a snow white amorphous powder, readily soluble in water. It is precipitated by 60% alcohol and according to Gautier by 36% alcohol in presence of salts. It is precipitated by $Ba(OH)_2$ and basic lead acetate. Heated with dilute nitric acid, it is converted into oxalic acid. Boiling with dilute mineral acids converts it into dextrose. Gautier says that for hydrolysis, it is necessary to heat it at 115°C with 5 or 6% acid and that the product has a reducing power slightly greater than dextrose. Several methods have been used for estimating glycogen, that suggested by Brücke[29] is probably as trustworthy as any. His method consists of digesting the meat with water on a steam bath, filtering, and then precipitating the proteid matter after acidifying with HCl with the double iodide of potassium and mercury. This precipitate is filtered off and the glycogen precipitated from the filtrate with alcohol of such strength as will make the solution contain 60% of absolute alcohol. The precipitate is filtered on a weighed filter, washed with 60% alcohol, then with 95% alcohol and finally with ether. The residue is dried at 115° 120°C and weighed.

Inosite ($C_6H_{12}O_6$ plus $2H_2O$) is a non-fermentable isomer of glucose. It is optically inactive and does not reduce metallic ox-

ides or Fehling's solution, although it turns the latter green. It crystallizes in large colorless rhombic crystals, which lose thier water at 110°C or if they are left exposed to the air, a long time. The crystals melt at 217°C. It is soluble in six parts of water and has a sweet taste. Slightly soluble in cold dilute alcohol and ether. It does not brown on boiling with dilute sulphuric acid.

Lactic acid. There are four lactic acids of the formula $C_3H_6O_3$. Hydracrylic acid is not found in the animal organism. Ethidene lactic acid has three modifications, the optically inactive fermentation acid, the dextro rotary paralactic or sarcolactic acid and the laevo rotary acid produced by the action of bacteria on alkaline solutions of cane, beet or milk sugar and glycerine. It is soluble in water alcohol and ether. It unites with dextro rotary lactic acid to form fermentation lactic acd. The water solution of its salts are dextro rotary. The sarco lactic acid is the one which is found in largest quantity in meat extracts. Liebig thought that sarco and fermentation lactic acids were identical. They are colorless or faintly yellow syrups, with an acid reaction, which mix in all proportions with water alcohol and ether. Their salts are soluble in water and most of them in alcohol. They are distinguished mainly by their optical activity, solubility and by the different amounts of water of crystallization of the calcum and zinc salts. The table following shows these differences.

Salt.	Acid.	Solubility.	Crystal water.
Zinc.	Fermentation	58-63 parts	3 molecules.
Zinc.	Sarcolactate	17.5 "	2 "
Ca.	Fermentation	9.5 "	5 "
Ca.	Sarcolactate	12.4 "	4-1/2"

For the quantitative estimation of lactic acid, Palm's method[30] has been used considerably. The method will be discussed later.

The Mineral Constituents of Muscle.

Liebig[31] says that the broth from meat contains soluble phosphates with alkali bases, lactic and inosinic acid in the form of salts, also magnesium phosphate with traces of calcium phosphate. In muscle the quantity of magnesium is about double the quantity of calcium. Another author in investigating the mineral constituents of flesh estimated the following:- potassium and sodium chloride, potassium sulphate, dipotassium phosphate, calcium and magnesium phosphates.[32] Hammersten says sulphates exist only in small quantities in muscle but are formed by burning the proteids of muscle and hence occur in abundance in the ash. Some investigators have determined the quantity of ferric oxide and sulphur.[33] Allen says that the salts of meat extract consist chiefly of earthy phosphates, potassium chloride and acid phosphate. Lactates and other organic salts of potassium are present. According to one author the mineral constituents of fresh meat are as follows based on 100 parts of meat:-

Soluble	KCl	.112.
	NaCl	.023.
	K_2SO_4	.055.
	HK_2PO_4	.409.
	$Ca_3(PO_4)_2$	.018.
	$Mg_3(PO_4)_2$	.036.
Insoluble	Ca & Mg Phosphates	.440.
	Total	1.093.

From the foregoing discussion it is very evident that even the

most detailed analyses yet made are only approximately accurate. Cautier's results, probably the best we have, are only averages of of the composition of the extract of a liter of broth. Even it shows extractive matter equaling more than 14%. This is a very indefinite term and may serve to cover the percentage of unknown material obtained by difference, although this is not always the case. In the following work, I have endeavored to separate the various constituents and arrive at a knowledge of the undetermined material and the quantities present.

Methods used in analysis. Total Solids. In the determination of total solids an aliquot portion of the solution was measured out by means of a pipette into a weighed platinum dish and evaporated to dryness on a water-bath. The dish was then transferred to a water oven and heated for periods of an hour or more at 100°C until constant in weight. Ash--The dry residue from the total solids determination was charred at a temperature below redness and then extracted with hot water. The precipitate was filtered on ashless filter and washed. The filter and contents was then ignited until colorless or nearly so. The acqueous solution was then added, evaporated to dryness on a water-bath and the residue ignited at low temperature until white or nearly so.

Total nitrogen--An aliquot portion of the solution was measured into suitable Kjeldahl flasks by means of a pipette. 25cc concentrated H_2SO_4 were added and .6 gram of mercury. The flasks were heated up gently at first and then strongly for at least two hours and longer if the solution was not clear at the end of that time. At the end of the digestion the contents were oxidized with a small

amount of powdered permanganate. When cool, 200cc of nitrogen free water, 80cc of strong alkali containing K_2S in solution, and a few granules of zinc were added and the ammonia distilled over and caught in measured quantities of standard HCl. The excess of acid was titrated with standard ammonia, using 1cc lacmoid as an indicator.

Nitrogen in proteids other than Peptones 50cc solution were acidified with 1cc H_2SO_4 (dilute) and saturated with powdered zinc sulphate at the ordinary temperature. The solution was allowed to stand over night and the precipitate filtered off, washed with a saturated solution of the precipitant and the nitrogen determined by the Kjeldahl method as described above. Peptone Nitrogen, The filtrate from the above determination was diluted with an equal volume of water, acidified with 5cc HCl (10%) and liquid bromine added a little at a time, with constant agitation, until the solution was saturated. It was washed with bromine water and the precipitate decanted off. Returned the filter and contents to the flask and determined the nitrogen as already described. Any other methods used in this work will be discussed as they come up.

Method of obtaining the Extract.

A piece of beef round weighing twelve pounds or more was obtained from the meat market. It was rather lean and contained no bone. The sample was freed from the remaining fat and gristle as far as possible. It was then cut into narrow strips, which were passed through the sausage mill. After passing through the mill a second time, the sample was divided into two lots and weighed. The total weight was 5478 grams. The two lots were placed separately in

large porcelain dishes and extracted with 2 1/2 liters of water. The chopped meat was mixed thoroughly with the water by means of the hands or by stirring with a large knife. After standing about half an hour, the mass was transferred to a large piece of previously boiled muslin which was stretched on a wooden frame. After draining fifteen or twenty minuites, the remaining portions of liquid were pressed out by means of the hands.

The third extract of the first lot was used for the second extraction of the second lot, etc. This process was continued until nine extractions had been made. The ninth extract was made up to 2 1/2 liters and 250 cc taken for examination. This was evaporated to dryness in a weighed platinum dish, and total solids determined. The residue from 250cc eq als .0489 gr., which for the total 2 1/2 liters equals .489 gr. The extraction was repeated four times and the total solids in 250cc again determined. The residue equals .0399 gr. or .399 gr. for the 2 1/2 liters. .399 gr. equals .01% of the total weight of the hashed meat. This was considered sufficiently complete. As soon as obtained the extract was evaporated down in large porcelain lined pans. The proteids which coagulated during the heating were filtered off on large muslin filters. The combined filtrates were evaporated until the volume was about 250cc. This material was placed in a flask and allowed to stand in a cool place for a number of days to see if anything would crystallize out. Labeled 1269. We next secured another piece of beef round weighing about twenty pounds. The fat was removed as before, and the lean meat sliced into narrow strips and ground. It was separated into three lots for extraction, the combined weights being 7725 grams.

This was extracted about ten times. Owing to an accident a small amount of the material was lost and hence quantitative results rendered inaccurate. We proceeded, however, as before and made the combined final residues up to one liter. Labeled 1270. This material was used in the preliminary investigation. In order to secure more material which could be used in quantitative work another piece of beef round weighing about eighteen pounds was secured. This was treated in the manner above described, the total weight of the hashed meat being 7202 grams. This was separated into two lots and extracted with three liters of water at a time, fifteen extractions being made. The last extract was quite clear and was made up to three liters. 250cc were taken for total solids, residue eq als .0717 gr. This was .011% of the total weight of the hashed meat, and was considered sufficiently complete. The combined extracts were evaporated down to about one liter. Labeled 1272.

Analytical work.

In beginning this work a preliminary experiment was made with sample 1270 which was obtained from 7725 grams of hashed meat.

The second lot was obtained from what was originally sample 1269 obtained from 5478 grams of meat plus sample 1272 obtained from 7202 grams of meat plus a quantity of material obtained by boiling about 20 pounds of meat for digestion experiment. As these analyses correspond somewhat closely, we shall carry them along together, making note of those digressions whech were made in each case.

The first lot will be designated by serial number 1270 and the second lot by 1310.

Sample #1270 was made up to a liter and 100 cc of this was diluted to 1000 cc. This was labeled 1270 B and used directly for analysis. The sample 1310 was made up to 1500 cc and fifty cubic contemeters of this were diluted to 500 cc and labeled 1310 A. In all cases, the percentage figures representing the analyses are calculated back to the total amount of the original sample. The analysis showed the following:

	#1270		#1310	
	Grams.	%	Grams	%
Solids.	444.13	100.00	652.56	100.00.
Ash.	106.89	24.06	152.04	23.29.
Nitrogen.	45.80	10.31	68.29	10.46.
Organic.	337.23	75.93	500.52	76.70.
Flesh Bases.	142.89	32.17	213.07	32.65.
Non-nitrogenous.	194.33	43.30	287.44	44.04.

These figures indicate that just about the ordinary % of ash or mineral matter is present in these extracts and that by far the largest portion is organic material. If we calculate the nitrogen to flesh bases by the factor 3.1?, it would appear that not quite half of this organic matter is nitrogenous. The problem which now lies before us is to determine the nature of this non-nitrogenous organic matter. Not that this will solve the whole problem but that this line of work promises important results. In order to get at this, it was decided that a method which would get rid of the mineral and nitrogenous constituents and concentrate the non-nitrogenous matter, would be of advantage. It has been known for many years that alcohol would precipitate a considerable portion of the proteid material as well as part of the salts. Bruylants (34) used a method of fractional precipitation as already stated. Micko states that the method works well. We have used this method and find that the non-nitrogenous organic matter does become concentrated as will be shown.

As the work upon 1270 B was only a preliminary experiment, we used only 200 cc for the alcohol precipitation, while in 1310 the entire lot minus 50 cc was evaporated to about 900cc and used. The 200 cc of 1270 were evaporated to 100 cc and an equal volume of alcohol was added, ~~to 1310~~. After standing over night, the precipitates were filtered off using the filter pump. The precipitates were washed with a solution of equal parts of alcohol and water.

The precipitate from 1270 was dissolved in 250 cc water and that from 1310 in 1000 cc. This latter was labeled 1310 B. 100 cc were taken for analysis and the remainder preserved for further use.

The solutions of the precipitates were analyzed and the results calculated to the original amounts are as follows:-

	1270 C a		1310 B	
	Grams.	%	Grams.	%
Total solids.	52.03	100.00	79.87	100.00.
Ash.	41.37	79.50	62.23	77.91.
Nitrogen.	.1285	.24	.3475	.43.
Organic.	10.65	20.47	17.64	22.09.
Flesh Bases.	00.40	.77	1.08	1.35.
Non-nitrogenous.	10.25	19.70	16.55	20.72.

We see from this table that just what we have been looking for has occurred, at least in part. Whereas in the original solution we had from 23-24% mineral matter, the first precipitate by alcohol contains from 77.9 to 79.5% ash. While the original solutions contained about 44 % non nitrogenous organic matter, the first precipitate contained only about 20% thus showing that it is largely left in solution. The nitrogenous constituents were not precipitated as fully as we anticipated. Before precipitating again, a part of the solution was removed from the filtrate from 1270 C_1a and analyzed. It was labeled 1270 C_2. The analysis showed:-

Total solids.	381.89 grams	100.00%.
Ash.	69.13 "	18.13%.
Nitrogen.	43.61 "	11.42%.
Organic.	312.75 "	81.88%.
Flesh Bases.	136.09 "	35.63%.
Non-nitrogenous.	176.66 "	46.25%.

This table only confirms the results given in the preceding one. To the filtrate from 1310 B_1 and the remainder from 1270 C_1A an

equal volume of alcohol (95%) was added, and after thoroughly shaking the mixture, was set aside over night. The supernatant liquids were then decanted off and the precipitates dark, heavy masses with a consistency of thick molasses, were washed with a solution of two parts of alcohol and one of water. They were then dissolved in water and analyzed.

	1270 C_3a.		1310 C_1.	
Constituents.	Grams.	%	Grams.	%
Total solids.	17.81	100.00	124.74	100.00.
Ash.	2.72	15.31	19.34	15.50.
Nitrogen.	2.00	11.27	2.73	2.19.
Organic.	15.08	84.68	105.39	84.49.
Flesh Bases.	6.26	35.16	8.54	6.85.
Non-nitrogenous.	8.82	49.52	96.85	77.64.

From this table there appears to be a wide difference in the result obtained by the same method of proceedure.

The only reason that I can see for this is the relative quantities used, that of 1310 C being almost four liters while 1270 C_3 was only about 300 cc. At any rate it appears that the method is accomplishing the object which we had in view, the mineral constituents having decreased from 24% to 15% and the non-nitrogenous organic matter increased from 43% to 49% in one case and from 44% to 77% in the other. Evidently these precipitates should present a rich field for examination. 1310 C_1 was preserved and examined later. The process was continued and an equal volume of alcohol added to the filtrates from the above precipitates. They were well shaken and left over night as before. Analysis showed the following:

	1270 C_4a.		1310 D.	
Constituents.	Grams.	%	Grams.	%
Total Solids.	92.29	100.00	68.214	100.00.
Ash.	17.05	18.48	11.15	16.34.
Nitrogen.	11.289	12.23	8.586	12.58.
Organic.	75.23	81.51	57.062	83.65.
Flesh Bases.	35.22	38.16	26.788	39.26.
Non-nitrogenous.	40.01	43.35	30.274	44.38.

Here the ash is higher again than in the preceeding precipitate and the non-nitrogenous matter is lower, while the quantity of nitrogen seems to remain more nearly constant, except in the case of 1310C_1 where it is extremely low.

To the filtrate from C_4a (600cc) an equal volume of 95% alcohol was added, the solution well shaken and set aside over night. The small precipitate which settled out was dissolved in water and analized. Labeled C_5a.

The alcohol was distilled from the mother liquor 1310D and the remaining solution concentrated to about 400 cc. Four volumes of alcohol (Sp. Gr. .825 at 73°F) were then added, the solution thoroughly mixed and set aside. This did not produce much of a precipitate so two volumes of 95% alcohol were added. A small quantity of precipitate settled out. The liquid was decanted off and the precipitate dissolved in water. This was labeled 1310 E and was kept for further study. An equal volume of alcohol was added to the mother liquor, the mixture, well shaken, labeled and set aside. A small precipitate, dark and thick settled out. The supernatant liquid was

decanted and the precipitate dissolved in water, labilling the same. 1310 F. This was added to 1310 E and the solution made up to 500 cc. Fifty cc were taken, made up to 250cc and used for analysis. Labeled 1310 E_2 & F_2.

	1270 C5a.		1310E_2 & F_2.	
Constituents.	Grams.	%	Grams.	%
Total solids.	20.438	100.00	39.195	100.00.
Ash.	4.716	23.07	6.465	16.49.
Nitrogen.	.537	2.62	4.973	12.68.
Organic.	15.72	76.91	32.729	83.50.
Flesh Bases.	1.677	8.20	15.516	39.57
Non-nitrogenous.	14.043	68.70	17.213	43.91.

In this case we have quite a wide difference again, the result for 1270 C_5a resembling somewhat the results obtained from the second alcoholic precipitate of the parallel experiment, 1310 C_1. The ash, however, is up almost to that of the original solution, the nitrogen has fallen almost 8% and the non-nitrogenous matter increased from 43% to 68%.

In 1310 E_2 & F_2 the results show very little deviation from the original, the ash is lower and the nitrogen about 2% higher.

From this point we shall be obliged to discuss the two series separately as the method of treatment was varied somewhat.

The solution from 1310 F was treated with an equal volume of alcohol. The total volume was almost 10 liters and had to be treated in three lots. A small quantity of a brown varnish like precipitate settled out. This was dissolved in water and made up to 250 cc. Labeled 1310 G_1 and analized.

Total solids.	5.5344 grams.	100.00%.
Ash.	.9930 "	17.94%.
Nitrogen.	.6765 "	12.22%.
Organic.	4.5414 "	82.05%.
Flesh Bases.	2.1106 "	38.13%.
Non-nitrogenous.	2.4308 "	43.92%.

If this table be compared with the two preceding ones, it will be noticed that the three are almost identical and hence no advantage has been gained by the last two precipitations. In the case of the sample 1270, however, it appears that the fourth precipitate contains the largest percentage of non-nitrogenous organic matter. I do not know why there should be any difference unless it is due to the quantities of material under treatment. The sample 1270 was treated as follows:-

The alcoholic solution remaining after the treatment described above; was evaporated to small volume and made up to 500 cc with nitrogen free water. This was labeled 1270 C_6a and analyzed.

Total solids.	260.3825 grams.	100.00%.
Ash.	52.1325 "	20.02%.
Nitrogen.	27.8000 "	10.67%.
Organic.	208.2500 "	79.98%.
Flesh Bases.	86.7360 "	33.31%.
Non-nitrogenous.	121.5140 "	46.66%.

This shows that the alcoholic solution after this treatment does not differ very much from the original solution and very little from

the solution which was analized immediately after the first precipitation. These facts lead me to believe that while the method employed adis in fractionating the constituents of meat extract, an indefinite continuance of the process would yield no practical advantage.

The remainder of 1270 C_6a was evaporated to a thick residue and treated with H_2SO_4 in sufficient quantity to convert all ash into sulphates. The material was then transferred to a flask and 750cc alcohol (95%) added. A rather heavy precipitate came down and this was filtered off, washed, dissolved in water, made up to 250cc, labeled 1270 C_7a and analized.

Total solids.	104.5582	grams.	100.00%.
Ash.	65.5083	"	62.65%.
Nitrogen.	5.6885	"	5.44%.
Organic.	39.0499	"	37.34%.
Flesh Bases.	17.7481	"	16.97%.
Non-nitrogenous.	21.3018	"	20.37%.

The result of converting the ash into sulphate may easily be seen since it has increased the amount of the ash precipitated in such a marked manner. The filtrate from 1270 C_7a was made up to a liter with 95% alcohol and treated as follows:-

25cc portions were titrated with $Ba(OH)_2$ solution using phenolphthalein as indicator. A took 28.75cc and B took 29.75cc. A sample of 250cc was heated to boiling, 287.5 cc $Ba)OH)_2$ solution added and the mixture allowed to stand over night, then filtered and the precipitate washed. The filtrate was evaporated to small volume in a porcelain dish on the water bath. A dark brown viscid residue re-

mained. This was dissolved in 250 cc water and analyzed. It was labeled 1270 C_8A_1.

Total solids.	222.82065	grams.	100.00%.
Ash.	81.5250	"	36.58%.
Nitrogen.	21.2384	"	9.53%.
Organic.	141.2956	"	63.41%.
Flesh Bases.	66.2638	"	29.73%.
Non-nitrogenous.	75.0318	"	33.63%.

By dissolving the ash in HCl and adding H_2SO_4 the excess of Baulum was determined. It was found that on a determination of 25cc out of 250cc, .0995 gr. of the ash out of .1661 gr. or about 3/5 was Barium. It was believed that a further separation of constituents could be made by treating this alcoholic solution with ether. Accordingly 500cc ether was added to 500cc of 1270 C_8A, the solution well shaken and set aside. A small precipitate settled out. The supernatent liquid was decanted, the precipitate washed, dissolved in 250cc water, and labeled, 1270 C_9B and analyzed. To the mother liquor more ether was added until the volume was two liters. A very slight precipitate settled out. The solution was diluted to 100cc, labeled 1270 $C_{10}B$ and analyzed.

	1270 C_9B.		1270 $C_{10}B$.	
Constituents.	Grams.	%	Grams.	%
Total solids.	61.7475	100.00	38.8975	100.00.
Ash.	1.2847	2.08	1.4332	3.68.
Nitrogen.	9.3285	15.10	5.5877	14.36.
Organic.	60.4628	97.92	37.4643	96.32.
Flesh Bases.	29.1049	47.29	17.4336	44.81.
Non-nitrogenous.	31.3579	50.78	20.0307	51.49.

From this it appears that ether would be a good precipitant for separating the non-nitrogenous organic matter, as 50% of the total was thrown down and very little ash with it. The greatest objection would be the small quantity of the precipitate obtained.

The filtrate from C_{10} B was distilled and the dark brown liquid residue dissolved in water, the solution neutralized with $BaCo_3$, filtered, made up to 250cc and analyzed. The results are:

Total solids.	64.1900 grams.	100.00%.
Ash.	19.3840 grams.	30.05%.
Nitrogen.	6.6780 grams.	10.35%.
Organic.	45.1060 grams.	69.95%.
Flesh Bases.	20.8353 grams.	32.30%.
Non-nitrogenous.	24.2707 grams.	37.63%.

Estimation of Proteoses.

For this determination 200cc of the solution were taken in duplicate, acidified with a few drops of H_2SO_4 (1-3) and $ZnSO_4$ added to saturation. The duplicates did not act in the same way. Filtered and washed with saturated solution of $ZnSO_4$. Sample A appeared the larger. Determined the nitrogen.

	In 200cc.	In liter original.	Proteoses.
A- -	.04988 Gr.N.	2.494 Gr.N.	15.587 Gr.
B- -	.03918 Gr.N.	1.959 Gr.N.	12.243 Gr.

Thus it appears that the method did not work very well, but probably this was due mainly to the lack of experience in manipulation.

Since it appears that quite a large percentage of the material of meat extracts determined by difference is organic matter containing little or no nitrogen it was thought that perhaps some clue could be obtained by estimating the reducing power of a definite quantity of extract. This seemed all the more probable since Miss K. I. Williams (35) obtained quite strong evidence of reducing substances in flesh of fish. For the determination we took 75cc of sample 1270 duplicate in 250cc flasks 10cc HCl (Sp. Gr. 1.125) were added and the solutions heated on a water bath three hours, using glass tubes four feet long for condensers. Removed the flasks from the water bath and neutralized the remaining acid with Na_2CO_3 and used 100cc in determinations.

Sample A gave .0173 gr. Cu_2O.

.0173 gr. Cu_2O equals .01535 gr. Cu.

.0153 gr. Cu corresponds to .0087 gr. dextrose.

.0087 x 2.5 x 13.33 x 10 equals 2.899 gr. reducing substances for the entire liter.

Sample B gave .0172 gr. Cu_2O.

0172 gr. Cu_2O equals .01526 gr. Cu.

.01526 gr. Cu corresponds to .0086 gr. dextrose.

.0086 x 2.5 x 13.33 x 10 equals 2.865 gr. reducing substances calculated to dextrose for the liter.

This total of 2.8 grams is a little over 0.6% of the total solids. In this experiment, however, the precipitate did not have the characteristic appearance of the usual copper precipitate. It was dark and flocculent and colored the solution green.

Determination of free and combined organic acids.

In speaking of the non-nitrogenous extractive matters, Allen[1] says that lactic acid and lactates probably predominate, but little is known concerning the quantities. He estimates that if lactic acid is present in fresh meat to 0.06% and if it takes 34 parts of meat to produce one part of extract, there should be 2.04% lactic acid in latter. This subject was studied at considerable length and the results obtained are here presented.

In order to determine the free acid 100cc of the original liter of 1270 was taken and evaporated to small volume. 100cc alcohol(95%) was added and the mixture well digested with stirring. Let stand over night. We filtered the supernatant liquid and washed the residue with 60% alcohol a number of times. We evaporated the filtrate to a syrup, treated with strong alcohol again and heated on water bath. The solution was cooled, 50cc more alcohol (95%) added and when supernatant liquid was clear, added filtrate. Washed the residue with strong alcohol. Evaporated the filtrates to a syrup, took up with water and extracted repeatedly with small quantities of ether. 500cc were used. The ether was distilled off and the residue treated with a little water and heated on the water bath. A few drops of phenolphalein were added and the soltuion titrated with N/10 NaOH 3.5 cc were required. The acidity was calculated to lactic acid which equaled-- .009 x 3.5 equals .0315 grams.
.0315 x 10 equals .315 grams lactic acid in the liter of original material.

In order to determine the combined acid the residues from the

[1] Allen. Com. Org. Anan. V. IV. p. 335.

above determination were treated with 5cc concentrated h_2SO_4 diluted to 10cc with distilled water. Extracted with alcohol as before and the residue was taken up with a very small quantity of water. Larger portions of ether were used in making the extractions this time. In all about twenty extractions were made. The ether extract on evaporation left a small quantity of sharp needle like crystals. These dissolved in water and oily globules of a dark brown color appeared. On the surface of the water transparent oily patches were visible. There was also present an amorphous sponge like solid. Titration of the solution. Phenoephthalein used as indicator.

A_1 192.5cc

A_2 9.5cc

A_3 4.0cc

A_4 2.5cc

A_5 3.5cc

A_6 1.2cc

Total 213.2cc N/10 NaOH.

After the first titration the solution was heated on the water bath five or ten minuites and then titrated again. This process was continued until upon heating the red color did not reappear.

The reason which is given for the titration appearing in this way is the presence in the solution of an anhydride which does not neutralize the alkali. Upon heating, however, the anhydride goes over slowly into the acid and may be titrated.

The solution was extracted again, the ether extract washed, distilled and the acid titrated after heating the solution to boiling. Total NaOH used equals 35.3cc. Total for these two series of ex-

tractions 248.5cc N/10 NaOH. In the following titrations, NaOH with a factor .00268 was used.

3rd series	15 extractions	required	93.0cc.
4th "	15	"	"	69.0cc.
5th "	15	"	"	41.5cc.
6th "	15	"	"	30.0cc.
7th "	15	"	"	24.0cc.
			Total.	257.5cc.

.009 x 248.5 x 10 equals 22.365 grams.

.00268 x 257.5 x 10 equals 6.901 "

Free acid. .315 "

Total. 29.581 "

$\frac{29.581 \times 100}{444.12} = 6.66\%$ acid.

Although this seems like a rational determination, we were not willing to conclude from this evidence alone that over 6% lactic acid was present in meat extract. It was thought that it was just possible that some sulphuric acid might have been present in the ethereal extract. In order to prove whether or not this was true, the following tests were made. All the extracts were evaporated down to small volume and filtered into a 500cc flask. Took 25cc for determining H_2SO_4. Neutralized with two drops HCl and added a little excess, in all .75cc. The solution was heated to boiling; 5cc $BaCl_2$ were added. A slight turbidity resulted. Heated the solution, filtered, washed, dried and weighed. $BaSO_4$ equals .0063 gr. .0063 x 20 equals .126gr., which equals .053 gr. H_2SO_4. To neutralize this requires .04326 grams NaOH or about 12 cc. As the solution required over 500 cc NaOH to titrate it, one can easily see that the quantity

of H_2SO_4 carried over is insignificant.

50cc of the above solution were placed in a distilling flask with 5cc H_2SO_4 1/1 and distilled with steam. For neutralization the

1st 100cc	distillate required	2.1cc	NaOH.
2nd 100cc	" "	0.9cc	"
3rd 100 cc(More or less)	"	1.5cc	"
4th 100cc	" "	0.5cc	"
	Total	5.0cc	"

Repeating using 10cc H_2SO_4 1/1.

1st 100cc	distillate required	0.9cc	NaOH.
2nd 100cc	" "	0.4cc	"
3rd 100cc	" "	0.2cc	"
4th & 5th 200cc	" "	0.3cc	"
6th & 7th 200cc	" "	0.15cc	"
	Total	1.95cc	"

These tests show the presence in very slight quantities of a volatile acid.

Some quantitative tests of the extracted solution are as follows:-

Reagent.	Medium.	Result.
$Pb(C_2H_3O_2)_2$.	very slightly acid.	Precipitate.
$AgNo_3$.	acid with HNo_3.	No precipitate.

Neutral lead acetate--heavy flocculent curdy precipitate.

$K_2Mn_2O_8$---		decolorized.
$CaCl_2$---	Alkaline.	slight flocculent precipitate.
Fe_2Cl_6 ---	slightly acid	darkens on heating.
$CuSO_4$ (dil)---		no precipitate.

H_2SO_4(con) plus evaporated residue chars.

The next question was to find a method for estimating quantitatively the amount of lactic acid in the solution of extract. This is a very difficult matter because as Liebig says, lactic acid does not form a single completely insoluble salt with any of the metals known. The method used in most cases consists of converting the acid into the zinc salt and extracting with alcohol. The lactate of inactive acid remains insoluble. The para-lactate, however, is soluble in alcohol and hence this method does not serve our purpose.

The method which apparently best suited our purpose was that of (36)
R. Palm. He says that when treated with lead acetate and alcoholic ammonia, lactic acid is completely thrown down as a heavy granular precipitate of the formula $3PbO, 2C_3H_6O_3$. His method is as follows: Extract the animal or vegetable material with ether having previously acidified with H_2SO_4. Evaporate the solution to a syrup and treat with water. The solution is precipitated with lead acetate to get rid of any proteids, and filtered. On adding more lead acetate, followed by alcoholic ammonia the lactate is thrown down free from foreign substances. The precipitate is washed with alcohol and the lactic acid estimated by loss on ignition. Apparently this method is simple but it was found that the alcoholic ammonia when added to a solution of lead acetate alone produced a white granular precipitate. The author says that an acqueous solution of ammonia will do this.

He suggests that both the lead acetate and the lactic acid solution be obtained in alcoholic solution before proceedure.

In order to avoid error a blank determination was run parallel to the main one. 100cc or 1/5 of the ethereal extract was used in

the determination.

A		Blank	
Wt. of Filter & P'p't. equals	1.6629.	Wt. of Filter & Ppt. equals	0.9357.
" " " "	.6547.	" " " "	.5743.
P'p't. "	1.0082.	P'p't. "	0.3614.
Wt. of Dish & PbO equals	36.9818.	Dish & PbO equals	29.8592.
" " " "	36.1445.	" "	29.5582.
PbO "	0.7773.	PbO "	0.3010.

.7773 -.3010 equals .4763 1.0082 - .4763 equals .5319.

$\frac{.5319 \times 5 \times 10 \times 100}{444.12}$ equals 5.08%.

Since this agrees very closely with the result obtained by direct titration of the extract with Na OH it seems that we are justified in concluding that in this sample of extract at least lactic acid and lactates are present to the extent of about 6%.

We shall now return to sample 1310 and discuss somewhat the treatment of the precipitates obtained by means of alcohol. After removing 100cc for analysis, the remainder (900cc) was evaporated to small volume. On standing, quite a crop of crystals separated. These were filtered off, washed and recrystallized repeatedly from boiling water 1310 B_1a. Weight of crystals equals 3.1901 gr. It was surmised that these crystals were KH_2PO_4 and tests were made to see if this were true.

Test for phosphoric acid--very heavy precipitate.

" " hydrochloric " --none.

" " sulphuric " --none.

" " sodium --none.

" " potassium --very strong.

.5058 gr. of the substance was ignited in a platinum dish. The

residue weighed .4371 gr. The loss was .0687 gr. or 13.58%. The theoretical loss of KH_2PO_4 equals 13.24%.

The mother liquor from the first crop of crystals was evaporated further and another crop of crystals separated. Labeled 1310B_1b Weight of crop equals 4.6866 gr. Tested for Na -- none. K --good test. .2494 gr. substance was ignited in platinum dish and residue weighed .2148 gr. Loss equals .0346 gr. or 13.47%.

The mother liquor from this crop of crystals was still further evaporated and a third crop of crystals was obtained. After purifying by recrystallization they were weighed. Weight equals 5.1801. .2751 gr. lost on ignition. .0385 gr. or 13.99%.

Another crop of crystals weighing 7.8089 gr. was obtained which showed a loss on ignition of 13.30%. Some of this salt was analyzed for phosphoric acid by converting it into the magnesium pyrophosphate.

	Salt.	$Mg_2P_2O_7$.	Phosphoric acid.	%.
A	.2592	.2115	.05907	22.7.
B	.1825	.1502	.04195	22.9.

Theoretical % of Phosphoric acid in KH_2PO_4 equals 22.7%. Several more crops of crystals have been obtained but have not been purified sufficiently to justify analysis. More than 30 grams of crystals were obtained in a practically pure condition, the analysis of which show conclusively that the phosphoric acid exists as the mono potassium salt and not as the dipotassium salt, as many authors believe.

Evidences of reducing substances were obtained by treating one of the solutions from this precipitate by the method described previously.

The study of the second precipitate was followed for a consid-

erable period. It will be noticed by referring to the precipitate labeled 1310 C_1 that it had a very high percentage of non-nitrogenous organic matter. We tried by the method of fractional precipitation with alcohol to separate this material from the other constituents and to obtain it in a form which would crystallize. A large number of fractions were obtained but no crystals of any sort could be obtained, so the process was abandoned. Lack of time prevented further investigation along this line.

We shall now return to the treatment of the mother liquor from 1310 G_1. The total solution was ten liters or more. This material was placed in a S & G. 2 liter flask, about a liter or a liter and a half at a time, and the alcohol distilled off through a long condenser and the distillate caught in large flasks. The residue after each distillation was poured into another flask. At this point, an accident occurred which caused the loss of about a liter of the alcoholic solution. We continued, however, and the results given below are for the remainder or about none liters of solution. When all the alcohol had been evaporated off the residue was made up to a liter and labeled 1310 H. 100cc were taken, made up to 250cc and analyzed and the solution labeled 1310 Ha. The remainder was filtered as the solution did not appear clear. 100cc more were taken- made up to 250cc, labeled 1310 Hb and analyzed.

The following figures represent the analysis:

	1310Ha		1310Hb	
Constituents.	Grams.	%	Grams.	%
Total solids.	275.600	100.00	272.810	100.00.
Ash.	58.715	21.30	54.620	20.02.
Nitrogen.	29.567	10.72	28.940	10.60.
Organic.	216.885	78.69	218.190	79.98.
Flesh Bases.	92.24[illegible]	33.47	89.292	32.73.
Non-nitrogenous.	124.637	45.22	128.898	47.24.

If this be compared with 1270 C_6a, it will be seen that the percentages of constituents in the two alcoholic solutions are almost identical, it is more surprising, however, to note that the percentage composition agrees so closely with that of the original solution.

In order to find more nearly the true percentage of flesh bases some of the solution was examined for proteoses and peptones. 50cc in duplicate were taken, 1cc H_2SO_4 (dilute) added and powdered $ZNSO_4$ added to saturation. The precipitate was filtered and washed. Determined nitrogen in precipitate by the Kjeldahl method. The average of the two equals .014988 gr.N or for the total liter .7494 gr.

The filtrate from the above determination was diluted, 5cc HCl (10% and liquid bromine added until it no longer dissolved on shaking. Filtered of the precipitate, washed and determined the nitrogen. Average of two determinations is .03501, or for the total liter 1.7506 Gr. N.

The sum of these .7494 and 1.7506 equals 2.5000 gr. N. By subtracting this amount from the total nitrogen 29.567 gr., we have 27.067 gr. which may rightly be calculated to flesh bases.

Precipitation by means of phospho molybdic acid was also tried on some of the solution 1310 Ha and Hb.

25cc were diluted with 25cc H_2SO_4(1-3) and the reagent added slowly 5cc at a time until no further precipitate was produced. After each addition the solution was thoroughly stirred and allowed to settle. About 70cc reagent were added to each sample. After standing six days, the precipitate was filtered off, washed with a dilute solution of the reagent and the nitrogen in the precipitate determined. After filtering and washing another slight precipitate came down. It was filtered off and the nitrogen determined.

The nitrogen in the solution was determined also. Results are for entire liter alcoholic solution. Total N is 29.567. Total N is 28.940.

	1310 H a		1310 H b	
	Grams	%	Grams	%
1st. Ppt.	7.084	24.89	6.884	24.73.
2nd Ppt.	.2747		.2747	
In solution.	19.615	66.34	20.2900	70.11.
Total.	26.9737		27.4480	

The remainder of 1310 H(800cc) was evaporated to small volume and an equal volume of alcohol added. 1310 K. No precipitate. About 100cc ether was added to this alcoholic solution in a separating funnel and well shaken. An alcoholic and ether layer separated. This was poured off and labelled 1310 K b.

The dark residue which remained 1310 K a was treated with 100cc ether and a second separation took place. Ethereal layer removed and labeled 1310 K a.

The acqueous solution which was again labeled 1310 K a was evap-

orated to small volume - made up to one liter.

We took 50cc made up to 250cc and used for analysis 1310 K x. Figures are for the entire liter of alcoholic extract.

Total solids.	223.375	100.00%.
Ash.	48.375	21.65%.
Nitrogen.	23.5093	10.52%.
Organic.	175.0000	78.34%.
Flesh Bases.	73.3490	32.83%.
Non-nitrogenous	101.6510	45.50%.

To 1310 K b an equal volume of ether was added. A considerable quantity of a light yellow colored liquid separated and was removed by a separating funnel. Labeled 1310 K b_1.

The second ethereal layer 1310 K a_1 which was obtained above was treated with an equal volume of ether. More light yellow liquid separated. This was removed and added to 1310 K b_1.

The residues from 1310 K a_1 and 1310 K b_1, we put together and added twice their volume of ether.. More yellow liquid separated and it also was removed and added to 1310 K b_1. The whole was now evaporated down to small volume, made up to 250cc labeled 1310 K C and analyzed. Results calculated to original liter labeled 1310 H.

Total solids.	30.9950	grams.	100.00%.
Ash.	.1175	"	0.37%.
Nitrogen.	3.4075	"	10.99%.
Organic.	30.8775	"	99.61%.
Flesh Bases.	10.6314	"	34.30%.
Non-nitrogenous.	20.2461	"	65.32%.

Tested the ash from this determination, $AgNO_3$, gave quite a heavy

precipitate, part of which blackened on standing in the light $(NH_4)_2 MoO_4$ in HNO_3 solution, gave only a very slight precipitate.

$BaCl_2$ in HCl solution gave good precipitate which was white. Qualitative tests showed presence of Na - K - and traces of Ca.

From this analysis, it appears that the material extracted by ether from an alcoholic solution of meat extract would serve excellently for the investigation of the organic matter present, there being only a fraction of a percent of mineral matter in the solution. The greatest disadvantage in a case of this kind is that when such a solution is obtained, the quantities are usually so small that it is difficult with the amount on hand to make a very detailed investigation.

Took 750cc of the residue from 1310 K a and added basic lead acetate in lots of 25cc until no further precipitate was produced. Filtered off the precipitate and treated as will be shown later. H_2S was passed through the filtrate, the PbS collected on a filter, washed with hot water, dried and kept. The filtrate was concentrated, made up to a liter, labeled 1310 M and analized. The results will be given below. The precipitate from the basic lead acetate was suspended in water and H_2S passed through the solution until it was thought that all had been decomposed. The PbS was filtered off, washed with hot water, dried and kept. The filtrate was concentrated to 500cc, labeled 1310 N and analyzed.

	1310 M.		1310 N.	
Constituents.	Grams.	%	Grams.	%
Total solids.	124.400	100.00	3.9950	100.00%.
Ash.	29.820	23.03	.4750	11.88%.
Nitrogen.	13.012	10.05	.3662	9.16%.
Organic.	99.640	76.96	3.5200	88.11%.
Flesh Bases.	40.5974	31.36	1.1425	28.59%.
Non-nitrogenous.	50.05	45.61	2.3775	59.51%.

I had expected that the basic lead acetate would precipitate almost if not quite all of the nitrogenous material, but from this last table, it appears that there is a larger percentage of nitrogen in the filtrate than in the precipitate.

We were prevented from carrying out the plans of this work any further for want of time.

In concluding, it may be well to make a brief summary of the results of this work.

First. It may be said that fractional precipitation by alcohol does serve the purpose of concentrating the non-nitrogenous organic matter in such a manner that it may be more readily separated and identified.

Second. The presence of substances which had a reducing action on Fehling's solution was proved beyond doubt, but the methods used to determine the amount of these substances could not be depended upon.

Third. The quantity of combined lactic acid or lactates was shown to be larger than has been supposed heretofore.

Fourth. By repeated recrystallization a pure salt was obtained

from the first alcoholic precipitate which proved by analysis to be mono potassium di hydrogen phosphate. This shows conclusively that while the dipotassium salt may be in the excess, yet a considerable percentage of the phosphoric acid is united in the form of the mono potassium salt.

Finally, I wish to express my gratitude to Dr. Grindly for the advice and assistance which he has given me during this work.

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